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Allele/haplotype variation in the MHC-DQA2 gene in Spanish sheep and its association with footrot susceptibility

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ABSTRACT

Footrot is a contagious disease that affects the hoof of sheep and other ungulates. The severity of the disease varies from a slight limp to the death of the individual due to injuries that prevent them from feeding. Variants of the Major Histocompatibility Complex (MHC)-DQA2 gene (MHC-*DQA2*) have been associated with a greater or lesser susceptibility to footrot in Greek, New Zealand and German sheep. In this study, variation in ovine MHC-*DQA2*, the absence or presence of footrot and the severity of infection was analysed in 117 Spanish Merino, Black Merino and Churra Lebrijana sheep. A total of 21 alleles/haplotypes and 65 genotypes were found with different frequencies in these breeds. As found in other studies, the MHC-*DQA2* allele *1101 appeared to be associated with increased susceptibility to footrot, while allele *1201 appeared to be associated with decreased susceptibility. Overall this would suggest the ovine MHC plays a role in controlling susceptibility to footrot infection and that there are breed differences in susceptibility. Sheep might therefore be able to be selected by their MHC-*DQA2* alleles/haplotypes to reduce the incidence of the disease in flocks.

1. Introduction

Footrot is a contagious hoof disease of sheep and other ungulates and begins as an interdigital dermatitis, which is followed by lesion development on the interdigital (axial) wall of the hoof and the subsequent separation of the hard horn from the foot (called under-running) (Bennett et al., 2009; Hickford et al., 2011). Footrot can cause also a reduction in feed intake, which results in a decreased quantity and quality of production, and in the most serious cases the death of the animal (Stewart, 1989).

The disease are associated with the infection of different bacterial being the *Dichelobacter nodosus* the essential transmitting agent (Beveridge, 1941; Dewhirst et al., 1990), although there are indications that different strains of *D. nodosus* may cause differences in the severity of the infection and should therefore be taken into account in eradication programs (Allworth, 2014).

Footrot is more prevalent in high rainfall regions, so it affects countries with the higher levels of rainfall. In that sense, the economic impact of footrot in UK is estimated to be 27,500,000-31,000,000 € per year (Nieuwhof and Bishop, 2005; Wassink et al., 2010). In fact, Winter and Green (2017), calculated that the overall cost of lameness per ewe and year in flocks with ≥10% lameness was approximately 7.45 € versus 4.50 € for flocks with <5% lameness.

In Spain, the economic losses might be around 14,000,000-18,000,000 € per annum, with it affecting several breeds (Martín, 2017), especially for those Spanish sheep breeds that are not accustomed to humid environments during the rainy seasons.

The Spanish Merino sheep (hereinafter referred to Merino in the text) is one of the most important sheep breeds globally and prized for its fine wool production. It is an indigenous breed, with an estimated census around two million reproductive females in 2020, whilst also being the origin of, or having a genetic influence over, a large number of sheep and breeds globally (Pedrosa et al., 2007). Like many other sheep breeds, the Merino is prone to footrot and this limits its use in wetter climates. In contrast, Churra Lebrijana sheep have been bred for many generations in the marshlands of the Andalusia Region of southern Spain, and they are reported to not get footrot (FAO, 2015).

The fight against this disease has traditionally been based on the sanitary management of the flock. Although vaccination has been used as a solution to control this disease (Ennen et al., 2009), but it has been showed to be less effective than foot-bathing as treatment (Allworth and Egerton, 2018).

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For decades, it has been known that there is a different genetic susceptibility within the same breed, without knowing the origin of said susceptibility until much more recently. Thus Raadsma et al. (1994) and later Nieuwhof et al. (2008) determined that the genetic susceptibility to this disease presented a medium heritability (15–25%), which allows the selection of more resistant candidate animals for breeding (Raadsma and Dhungyel, 2013b).

The determination of a greater or lesser susceptibility to the footrot of genetic origin linked to loci of the MHC system (Hickford et al., 2004) has opened the possibility of approaching the fight against the disease from the genetic point of view in a more efficient way.

The Major Histocompatibility Complex (MHC) has been the focus of study to better understand susceptibility to infectious diseases in several livestock species, including sheep (Scott et al., 1991; Hickford et al., 2011). Of the various MHC loci, the DQA2 gene (*DQA2*) has been reported to be associated with variation in susceptibility to footrot in Corriedale sheep (Escayg et al., 1997), Chios sheep (Gelasakis et al., 2013) and German Mutton Merino, German Merino and German Blackheaded Mutton sheep (Hickford et al., 2007; Ennen et al., 2009). This gene has not been investigated in Spanish sheep with footrot, and accordingly the aim of this study is to determine whether an association exists between the presence/absence of footrot and variation in *DQA2* in three Spanish sheep breeds (Spanish Merino, Black Merino, and Churra Lebrijana breed).

2. Materials and methods

2.1. Sheep selection, blood sample collection and DNA purification

A total of 117 sheep from three breeds were selected for the investigation. These comprised 92 animals diagnosed with footrot, consisted of 65 Merino sheep from two different flocks from the south of Spain and 27 Churra Lebrijana sheep from two different flocks raised in Andalusian marshland ("marisma") in the south of Spain. The age of the sheep ranged between 2 and 7 years. In order to analyse the allelic frequency of the DQA2 gene in the Black Merino sheep, the remaining 25 sheep, belonging to a two different flocks, were selected randomly from a DNA bank. All of the 117 sheep were farmed in an extensive production system in a region that gets between 450 and 600 millimetres of rainfall per year (Merino and Black Merino), and 800 to 1000 (Churra Lebrijana).

A blood sample from each sheep was collected onto TFN paper (Munktell Filter AB, Sweden) and genomic DNA was purified using a two-step washing procedure, as described in Zhou et al. (2006).

2.2. Footrot diagnosis

The footrot diagnosis was carried out on the 92 sheep in 2017 a particularly rainy year in the flock locations, with an average rainfall of 30.00% above normal, with episodes of extreme rains during the spring months. This allowed the sheep with the disease to present very obvious symptoms enabling simple diagnosis using visual assessment and hoof inspection. The diagnosis of the presence or absence of footrot and symptoms was undertaken by a clinical veterinary specialist in this disease. The sheep were classified into three different groups based on their hoof condition. 'Healthy', being sheep with no evidence of lameness or damage to their hooves; 'slight limp', being sheep that had a mild limp, but did not display evidence of any kind of external hoof damage; and 'limp', when the animals displayed a severe limp and had clear under-running of the hoof.

2.3. DQA2 genotyping

The DQA2 gene was genotyped using the PCR-single stranded conformational polymorphism (SSCP) technique described by Hickford et al. (2004). Briefly, the polymorphic exon 2 of the ovine DQA2 gene was amplified using *DQA2*-specific primers (5′-actaccaatctcatggtccctct-3′ and 5′-ggagtagaatggtggacacttacc-3′). The *DQA2*-specific primers amplify both *DQA2* and *DQA2*-like sequences (Hickford et al., 2004), and to date no one has described a means of differentiating the two loci. Moreover, the term "*DQA2*-haplotype" or "haplotype" was used to represent a *DQA2*-allele accompanied by an additional *DQA2*-like sequence (Hickford et al., 2007; Ennen et al., 2009). Amplification was carried out in a 15-μL reaction containing the genomic DNA on one 1.2-mm punch of TFN paper, 0.25 mM of each primer, 150 mM dNTPs (Eppendorf, Hamburg, Germany), 0.5 U Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× the reaction buffer (containing 1.5 mM $MgCl_2$). After an initial denaturation at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s were utilised, followed by a final 5 min of extension at 72 °C.

The amplicons produced were analysed using Single Strand Conformation Polymorphism (SSCP) in 14% acrylamide:bisacrylamide (37.5:1; Bio-Rad, Hercules, CA) gels. Briefly, a 0.7-μL aliquot of each amplimer was mixed with 7 μL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95 °C for 5 min, the samples were cooled rapidly on wet ice and then loaded onto the gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad) for 18 h in 0.5× TBE at 5 °C and 380 V. The gels were silver-stained according to the method described by Byun et al. (2009). Amplicons representative of known *DQA2* sequences were also included in each polyacrylamide gel and their banding patterns were used as standards for determining the alleles/haplotypes present in the individual sheep. Once the genotypes were determined, genotypic and allelic frequencies were calculated.

2.4. Statistical analyses and footrot gene-marker scores

Association of alleles/haplotypes frequencies in affected and non-affected groups of the Merino and Churra Lebrijana breeds were performed using the Maximum-Likelihood Chi-square test in a first approximation. A Logistic Regression model was then employed to give a more specific analysis to determine whether each allele was found more commonly in affected or non-affected sheep.

3. Results and discussion

Although Egerton and Raadsma (1991), described breed differences in resistance to footrot, results were not clear to be due the breed or the sire effect. However, industry observations suggested that British breeds of sheep tend to have greater resistance to footrot than wool breeds such and Merino or Merino derived breeds (Raadsma and Dhungyel, 2013b).

Later, the study of footrot has been addressed in multiple works about genetic resistance to diseases in sheep, as well as the different ways of eradication (Bishop and Morris, 2007; Raadsma and Egerton, 2013; Raadsma and Dhungyel, 2013a; Allworth, 2014; Azarpajouh et al., 2019).

This paper describes the association of genetic variants of the MHC-DQA2 gene with susceptibility to footrot in the Spanish Merino sheep breed, and Churra Lebrijana breed. Additionally, the genotype for the *DQA2* gene has also been analysed in the Black Merino sheep. This breed is closely related to the Merino breed, being considered the ancestral population of the Spanish Merino. It was included in the genetic analysis, to determine if the frequency of the most sensitive or resistant alleles was equal to that of the current merino population.

The clinical veterinary diagnosis results suggest that none of the animals of the Churra Lebrijana breed were affected with footrot, as might be expected, given they have been described as showing resistant or tolerance to footrot by the FAO (FAO, 2015). This rare breed's usual habitat is the marsh or marisma of southern Spain, a region being characterised by large areas of land that are regularly prone to flooding. One of the most important extrinsic predisposing factors to the development of footrot infections is the soil moisture as this can soften or

Table 1
DQA2 allele/haplotype frequencies in sheep from different Spanish breeds.

Allele/ haplotype	Churra Lebrijana Non-affected ($n = 27$)	Merino			Black Merino ($n = 25$)
		Overall ($n = 65$)	Non-affected ($n = 44$)	Affected ($n = 21$)	
*0103	1.9	17.7	14.8	23.8	4.0
*0301	5.6	3.1	1.1	7.1	2.0
*0501	–	9.2	10.2	7.1	4.0
*0601	1.9	8.5	8.0	9.5	–
*0602	35.2	8.5	10.2	4.8	18.0
*08011	–	2.3	1.1	4.8	–
*0901	–	1.5	2.3	–	–
*1001	5.6	3.9	5.7	–	2.0
*1101	1.9	12.3	12.5	11.9	8.0
*1201	38.9	7.7	8.0	7.1	8.0
*0101-1401	1.9	6.9	8.0	4.8	28.0
*0101-1601	–	2.3	1.1	4.8	–
*0102-1401	–	0.8	1.1	–	–
*0401-1401	–	7.7	5.9	11.9	–
*0401-1501	7.4	0.8	1.1	–	2.0
*0402-1701	–	0.8	1.1	–	–
*0603-1101	–	–	–	–	4.0
*0701-1401	–	–	–	–	2.0
*0701-1601	–	0.8	1.1	–	–
*0702-1401	–	3.9	4.6	2.4	4.0
*0702-1601	–	–	–	–	12.0
*08012-0201	–	1.5	2.3	–	2.0

weaken the hoof, facilitating infection (Hurtado et al., 1998).

From the 65 Merino sheep studied, 21 were affected with differing degrees of footrot severity. The overall prevalence in the footrot exposed Merino sheep was 31.0% (43.0% and 24.0% in each flock respectively). Of the affected Merinos, five had a slight limp while the remaining 16 had a severe limp with damaged hooves.

Globally, a total of 21 alleles of *DQA2* and 65 genotypes were detected (Table 1) in the 117 sheep genotyped (9 alleles in Churra Lebrijana, 19 alleles in the Merino and 14 alleles in the Black Merino). In the Churra Lebrijana sheep, the frequencies ranged from 38.9% to 1.9%; in the Merino sheep they ranged from 17.7% to 0.8% and in the Black Merino sheep they ranged from 28.0% to 2.0%. The most common alleles/haplotypes in the Spanish sheep studied were *1201 and *0602 with overall frequencies of 38.9% and 35.2% respectively in the Churra Lebrijana sheep. Alleles/haplotypes *0101-*1401 and *0602 with frequencies of 28.0% and 18.0% respectively, were most common in the Black Merino sheep; and alleles *0103 and *1101 with frequencies of 17.7% and 12.3% respectively, were most common in the Merino sheep.

These figures are consistent with previous studies of *DQA2* allele/haplotype frequencies in Merino, Corriedale and Romney sheep (Hickford et al., 2007), where the most common *DQA2* - *DQA2*-like haplotype was *0101–1401 in all three breeds, and with *0102–1601 also being common (14.02%) in the New Zealand Romney sheep. In these sheep the DQA2 allele *1201 had an overall frequency of 15.76%. Alleles *0103 and *0602 (25.9% and 15.9%, respectively) were found to be most common in German Mutton Merino, German Merino and German Blackhead Mutton Sheep (Ennen et al., 2009) and in Chios dairy sheep the most common allele was *0301 with a frequency of 31.7% (Gelasakis et al., 2013).

In their study of the association between MHC variation and footrot, Escayg et al. (1997) revealed *DQA2* allele *1201 ('H') to be associated with decreased susceptibility and *DQA2* *1101 ('E') to be associated with increased susceptibility to footrot in one half-sib family of the Broomfield Corriedale sheep. Along with the Churra Lebrijana sheep, these Corriedale sheep have been identified in Table 1E11 of the FAO's 'Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture' (FAO, 2015) as showing 'resistance or tolerance to footrot'. It is therefore quite notable that allele *1201 (38.9%) was the most common in the Churra Lebrijana sheep, while allele *1101 was only found in one (1.9%) of these sheep. These data reinforce the hypothesis in the wider literature that there are some alleles of the DQA2 gene belonging to the MHC system that are associated with a greater or lesser resistance to footrot and that therefore could be selectable to the footrot genetic control.

In the same way, Ennen et al. (2009) found associations between *DQA2* and susceptibility to footrot, revealing that the likelihood of a footrot infection is lower for ewes having one of the *DQA2 – DQA2-like* haplotypes *0101-1401 (rare in the Churra Lebrijana sheep) and *0702-1401 (not found in the Churra Lubrijana sheep), than for ewes carrying the alleles *1101 and *0501. Their result for allele *1101 is consistent with the findings of Escayg et al. (1997) and with the low frequency of occurrence of this allele in the Churra Lebrijana sheep (1.9%). Allele *1101 occurs quite frequently in the Merino sheep (with a frequency of 12.5% in the non-affected Merino sheep and 11.9% in the footrot affected ewes). The allele was slightly less common (8.0%) in the non-affected Black Merino sheep.

Gelasakis et al. (2013) also revealed allele *1101 to be associated with increased susceptibility to footrot. A single copy of *1101 was associated with 9.0% higher susceptibility to footrot, whereas the presence of two alleles increased the probability of infection to nearly 34.0%.

Taken together with the results presented here, and the findings of Escayg et al. (1997) and Ennen et al. (2009), the case for allele *1101 being associated with increased susceptibility to footrot is compelling. The result is less convincing for *1201 being associated with increased resilience to footrot, albeit it was present at a high frequency in the Churra Lebrijana sheep, and was associated with resistance in the Escayg et al. (1997) study. This allele was not common in the Chios sheep (Gelasakis et al., 2013) or the German sheep (0.05%; Ennen et al., 2009). Care needs to be taken with this interpretation though, as the number of Churra Lebrijana sheep studied was small ($n = 27$), and in consequence the number of alleles identified (nine) was lesser. While it has been argued by (Calvo et al., 2011) that the Churra Lebrijana population is a rich reservoir of genetic diversity, they also acknowledge that the sheep are isolated from the other Churra-type breeds, it is considered an endangered breed with extreme risk and have the genetic signature of having had a demographic bottleneck, an observation that is perhaps supported by the low number of *DQA2* alleles/haplotypes that were found.

While alleles *0103, *0301, *0602, *1101, *1201, and haplotype *0101-1401 appeared in all the sheep studied here, alleles *0901 and *1001, and haplotypes *0102-1401, *0401-*1501, *0402-1701, *0701-1601 and *08012-0201 did not appear in the affected Merino sheep, despite being present in the other challenged (non-affected) Merino sheep. Allele *0601 was not found in the Black Merino sheep, and *08011 and *0401-*1401 were not recorded in the Black Merino and Churra Lebrijana breeds. According to Gelasakis et al. (2013), the *0101-1401 and *0702-1401 haplotypes also favoured footrot resistance. Haplotype *0101-1401 was more common in the non-affected Merino sheep than the affected Merino sheep, but was rare in the Churra Lubrijana sheep and common in the footrot unchallenged Black Merino sheep. Haplotype *0702-1401 was also more common in the non-affected sheep, but was not present in the Churra Lubrijana sheep. Gelasakis et al. (2013) also suggested that allele *0501 was associated with increased susceptibility to footrot, and it is therefore notable that it was not found in the Churra Lubrijana sheep.

It is remarkable, the difference found in the *1010-1401* allele, associated with the footrot resistance, between Black Spanish Merino (28%) and in the White Spanish Merino (6.9%). In the other hand, the allele *0501* associated with susceptibility to the disease, was found in the White Spanish Merino (9.2%) in more than a double proportion that in the Black Spanish Merino (4.0%). Those differences in allele frequencies could drive how resistant to footrot each breed appears.

When the affected sheep were compared with the non-affected sheep using a Maximum Likelihood Chi-square test, statistically significant

Table 2
Maximum likelihood Chi-Square test of affected versus non-affected sheep groups from Merino and Churra Lebrijana breeds.

	$MLChi^2$	p-value
DQA2 alleles/haplotypes	35.22	0.0089
DQA2 genotypes	75.61	0.0112

Table 3
Association levels of footrot alleles using Chi-Square decomposition of affected versus non-affected sheep groups from Merino and Churra Lebrijana breeds.

Allele/haplotype	Non-affected	Affected
*0103	−4.52	4.52
*0301	−1.40	1.40
*0501	−0.26	0.26
*0601	−1.26	1.26
*0602	4.85	−4.85
*08011	−1.32	1.32
*0901	0.46	−0.46
*1001	1.83	−1.83
*1101	−1.12	1.12
*1201	4.08	−4.08
*0101-1401	0.28	−0.28
*0101-1601	−1.32	1.32
*0102-1401	0.23	−0.23
*0401-1401	−2.72	2.72
*0401-1501	1.14	−1.14
*0402-1701	0.23	−0.23
*0701-1601	0.23	−0.23
*0702-1401	0.14	−0.14
*08012-0201	0.46	−0.46

differences between the alleles and the classification of the sheep as affected and non-affected were found (Table 2). Table 3 reveals the level of association of alleles with being footrot affected using a Chi2 decomposition with *Logistic Regression model*. Alleles/haplotypes *0602, *1001, *1201 and *0401-1501 are more frequently found in the footrot un-affected sheep, while *0103, *0301, *0601, *1101, *08011, *0101-1601 and *0401-1401 are more frequently found on the footrot affected group (Fig. 1).

This is the first preliminary work that approaches the study of the different alleles/haplotypes of the DQA2 gene and their distribution within several Spanish sheep breeds.

Although the results should be taken with some caution, analysing the environment effect it should be noted that, in the case of the Churra Lebrijana, linked to very humid environments, the incidence of footrot is null. On the other hand, the Merina is very cosmopolitan, being present in a multitude of environments. In our case, samples were obtained from 2 flocks from different areas (middle-mountain and plain) within the same region where water accumulation varies considerably. No differences were found between the frequency of animals affected and the level of the injuries in the affected ewes of each flock per breed. It was also possible to rule out the possible association due to the sire effect since the animals of these breeds belonged to herds with unrelated sires.

The sample size must be taken into account when interpreting the results, since in small populations the presence or absence of certain alleles can be random, causing some variants to be overrepresented and others to appear in low proportion (or even absent). This limitation is, in part, due to the scarce census of breeds such as the Churra Lebrijana, which is in extreme danger of extinction and whose population currently consists of only 68 animals (60 females and 8 males) distributed in 2 herds.

Allele associated to footrot resistance or susceptibility could be used like a selection criteria to the genetic improvement of sheeps. In the case of the Churra Lebrijana, the selection of more resistant animals does not make sense because the alleles associated with susceptibility to footrot are found at a low frequency, and the extreme vulnerability of this breed does not make possible a selective pressure that could lead to a loss of variability. In the case of the Black Merino breed, although it is also considered to be at risk of extinction, its census would allow the inclusion of resistance alleles as selection criteria. Both in Black Merino and Merino, it would be necessary to consider the frequency of the resistance allele to see if it is worthwhile or not to include it as selection criteria especially in those farms that are in areas with higher rainfall.

Nevertheless, the present study lays the groundwork for future work in which it would be interesting to increase the number of individuals per breed to obtain a more solid population size, and the number of breeds to obtain a more complete representation of the Spanish ovine

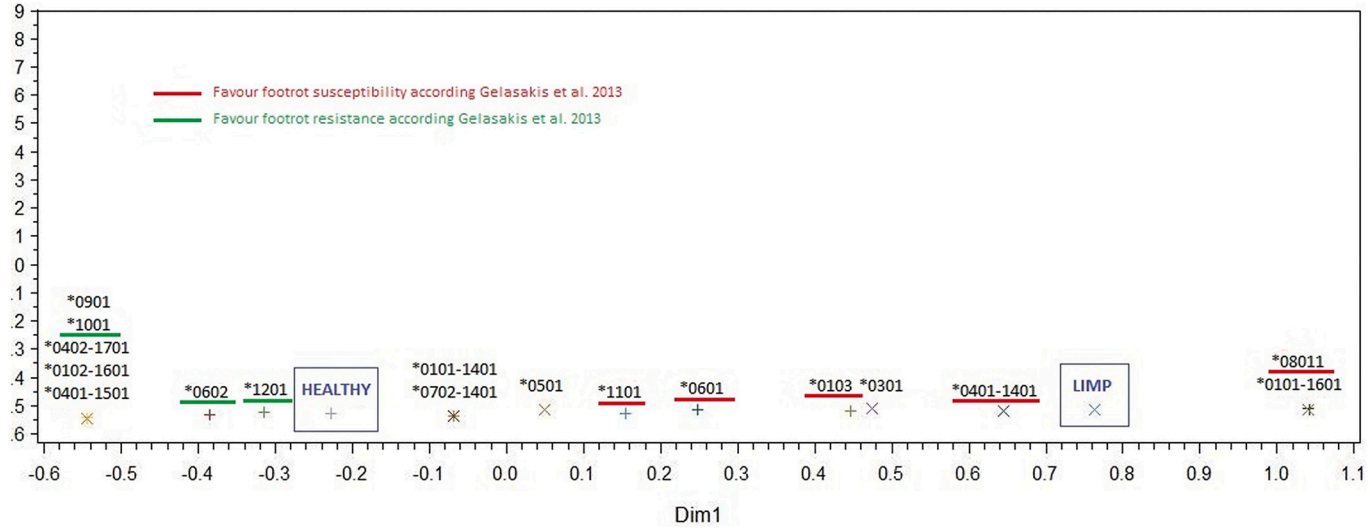

Fig. 1. *DQA2* allele/haplotype representation of the different Spanish breeds and comparison with the association detected by Gelasakis et al., 2013. Animals grouped by healthy (not affected by footrot) and limp (affected by footrot) are represented in the figure. Also the different DQA2 allele/haplotypes found in the animals analysed of the Spanish Merino, Spanish Black Merino and Churra Lebrijana breeds are represented in the figure in the relative position to the healthy and limp groups, according the allele frequency of each of them (those with higher frequencies being closer to each LIMP or HEALTHY group). In order to compare the results of the present work whit the results found in previous studies, the alleles associated with footrot resistance and those associated with footrot susceptibility according Gelakasy et al. 2013 were underlined in green and red respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

population.

4. Conclusion

In conclusion, genetic resistance to footrot was observed for the Churra Lebrijana sheep. As in other studies the MHC DQA2 allele *1101 appears to predispose to the disease, while other alleles are possibly associated with increased resistance. Overall this would suggest the ovine MHC plays an important role in susceptibility to footrot infection and that there are breed difference in susceptibility. Sheep might therefore be able to be selected by their *DQA2* alleles/haplotypes to reduce the incidence of the disease in flocks.

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Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper.

References

Allworth, M.B., 2014. Challenges in ovine footrot control. Small Rumin. Res. 118, 110–113.
Allworth, M.B., Egerton, J.R., 2018. Comparison of footbathing and vaccination to control ovine footrot in an experimentally infected flock. Aust. Vet. J. 96, 395–399.
Azarpajouh, S., Díaz, J.C., Clifford-Rathert, C., Correa, J.S., Lamberson, W., 2019. Genetics of footrot resistance in small ruminants. CAB Rev. 14.
Bennett, G., Hickford, J., Sedcole, R., Zhou, H., 2009. Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe 15, 173–176.
Beveridge, W.I.B., 1941. Foot-rot in Sheep, a Transmissible Disease due to Infection with Fusiformis nodesus (n. sp.): Studies on its Cause, Epidemiology and Control/by W.I. B. Beveridge. Council for Scientific and Industrial Research, Melbourne.
Bishop, S.C., Morris, C.A., 2007. Genetics of disease resistance in sheep and goats. Small Rumin. Res. 70, 48–59.
Byun, S.O., Fang, Q., Zhou, H., Hickford, J.G., 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Anal. Biochem. 385, 174–175.
Calvo, J.H., Alvarez-Rodriguez, J., Marcos-Carcavilla, A., Serrano, M., Sanz, A., 2011. Genetic diversity in the Churra tensina and Churra lebrijana endangered Spanish sheep breeds and relationship with other Churra group breeds and Spanish mouflon. Small Rumin. Res. 95, 34–39.
Dewhirst, F.E., Paster, B.J., La Fontaine, S., Rood, J.I., 1990. Transfer of *Kingella indologenes* (Snell and Lapage 1976) to the genus Suttonella gen. nov. as *Suttonella indologenes* comb. nov.; transfer of *Bacteroides nodosus* (Beveridge 1941) to the genus Dichelobacter gen. nov. as *Dichelobacter nodosus* comb. nov.; and assignment of the genera Cardiobacterium, Dichelobacter, and Suttonella to Cardiobacteriaceae fam. nov. in the gamma division of Proteobacteria on the basis of 16S rRNA sequence comparisons. Int. J. Syst. Bacteriol. 40, 426–433.
Egerton, J.R., Raadsma, H.W., 1991. Footrot. Breeding for Disease Resistance in Farm Animals. C.A.B. International, Wallingford, pp. 347–370.
Ennen, S., Hamann, H., Distl, O., Hickford, J., Zhou, H., Ganter, M., 2009. A field trial to control ovine footrot via vaccination and genetic markers. Small Rumin. Res. 86, 22–25.
Escayg, A.P., Hickford, J.G.H., Bullock, D.W., 1997. Association between alleles of the ovine major histocompatibility complex and resistance to footrot. Res. Vet. Sci. 63, 283–287.
FAO, 2015. Domestic Animal Diversity Information System (DAD-IS). URL http://dad.fao.org/.
Gelasakis, A.I., Arsenos, G., Hickford, J., Zhou, H., Psifidi, A., Valergakis, G.E., Banos, G., 2013. Polymorphism of the MHC-DQA2 gene in the Chios dairy sheep population and its association with footrot. Livest. Sci. 153, 56–59.
Hickford, J.G., Zhou, H., Slow, S., Fang, Q., 2004. Diversity of the ovine DQA2 gene. J. Anim. Sci. 82, 1553–1563.
Hickford, J.G., Forrest, R.H., Zhou, H., Fang, Q., Frampton, C.M., 2011. Association between variation in faecal egg count for a mixed field-challenge of nematode parasites and ovine MHC-DQA2 polymorphism. Vet. Immunol. Immunopathol. 144, 312–320.
Hickford, J.G.H., Zhou, H., Fang, Q., 2007. Haplotype analysis of the DQA genes in sheep: evidence supporting recombination between the loci1. J. Anim. Sci. 85, 577–582.
Hurtado, M.A., Píriz, S., Valle, J., Jimenez, R., Vadillo, S., 1998. Aetiology of ovine footrot in Spain. Vet. Rec. 142, 60–63.
Martín, P., 2017. El pedero en ovino. Tierras. Ovino 20, 58–64.
Nieuwhof, G.J., Bishop, S.C., 2005. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. Anim. Sci. 81, 23–29.
Nieuwhof, G.J., Conington, J., Bunger, L., Haresign, W., Bishop, S.C., 2008. Genetic and phenotypic aspects of foot lesion scores in sheep of different breeds and ages. Animal 2, 1289–1296.
Pedrosa, S., Arranz, J.J., Brito, N., Molina, A., San, Primitivo F., Bayón, Y., 2007. Mitochondrial diversity and the origin of Iberian sheep. Genet. Sel. Evol. 39, 91–103.
Raadsma, H.W., Dhungyel, O.P., 2013a. A review of footrot in sheep: new approaches for control of virulent footrot. Livest. Sci. 156, 115–125.
Raadsma, H.W., Dhungyel, O.P., 2013b. A review of footrot in sheep: new approaches for control of virulent footrot. Livest. Sci. 156, 115–125.
Raadsma, H.W., Egerton, J.R., 2013. A review of footrot in sheep: Aetiology, risk factors and control methods. Livest. Sci. 156, 106–114.
Raadsma, H.W., Egerton, J.R., Wood, D., Kristo, C., Nicholas, F.W., 1994. Disease resistance in Merino sheep. III. Genetic variation in resistance to footrot following challenge and subsequent vaccination with an homologous rDNA pilus vaccine under both induced and natural conditions. J. Anim. Breed. Genet. 111, 367–390.
Scott, P.C., Gogolin-Ewens, K.J., Adams, T.E., Brandon, M.R., 1991. Nucleotide sequence, polymorphism, and evolution of ovine MHC class II DQA genes. Immunogenetics 34, 69–79.
Stewart, D.J., Egerton, J.R., Yong, W.K., Riffkin, G.G., 1989. Footrot of sheep. Footrot and Foot Abscess of Ruminants. CRC Press, Boca Raton, pp. 5–45.
Wassink, G.J., King, E.M., Grogono-Thomas, R., Brown, J.C., Moore, L.J., Green, L.E., 2010. A within farm clinical trial to compare two treatments (parenteral antibacterials and hoof trimming) for sheep lame with footrot. Prevent. Vet. Med. 96, 93–103.
Winter, J.R., Green, L.E., 2017. Cost-benefit analysis of management practices for ewes lame with footrot. Vet. J. 220, 1–6.
Zhou, H., Hickford, J.G., Fang, Q., 2006. A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. Anal. Biochem. 354, 159–161.